ANTIGEN-ANTIBODY ANALYSIS IN LEISHMANIASIS

FINAL REPORT

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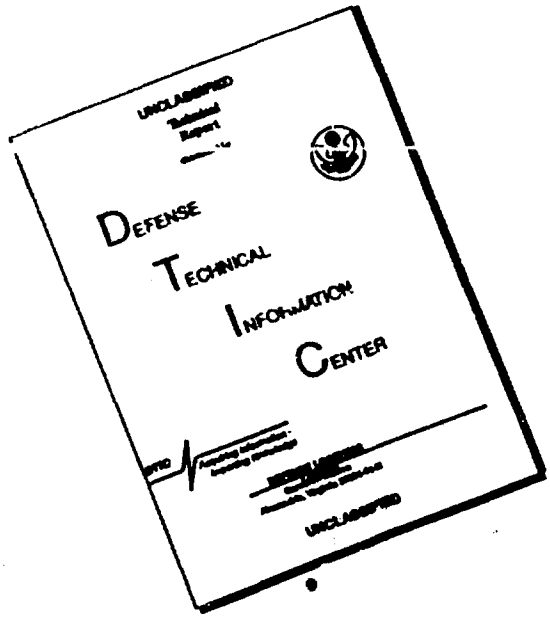

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Studies were performed to identify antigens of various species of New World Leishmania and Trypanosoma cruzi which would be useful in the differential diagnosis of diseases in humans caused by these pathogenic protozoa. Squirrel monkeys (Saimiri sciureus) were infected with Leishmania braziliensis or L. panamensis and/or T. cruzi and the levels and specificity of antibodies to antigens monitored during the course of single or sequential infections. Control sera were obtained from the monkeys prior to initiation of experimental infections. Antibody levels were determined by ELISA and the specificity for specific antigens of antibodies during the course of infections monitored by Western Blot analyses. Antigens of particular interest and potential use in immunodiagnostic assays were identified. Other information on the course of disease in the squirrel monkey model was determined and are discussed in this report.

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SUMMARY

Studies were performed to identify antigens of various species of New World Leishmania and *Trypanosoma cruzi* which would be useful in the differential diagnosis of diseases in humans caused by these pathogenic protozoa. Squirrel monkeys (*Saimiri sciureus*) were infected with *Leishmania braziliensis* or *L. panamensis* and/or *T cruzi* and the levels and specificity of antibodies to antigens monitored during the course of single or sequential infections. Control sera were obtained from the monkeys prior to initiation of experimental infections. Antibody levels were determined by ELISA and the specificity for specific antigens of antibodies during the course of infections monitored by Western Blot analyses. Antigens of particular interest and potential use in immunodiagnostic assays were identified. Other information on the course of disease in the squirrel monkey model was also determined and will be discussed in this report.

FOREWORD

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources Commission of Life Sciences, National Research Council (NIH Publication No. 86-23, Revised 1985).

Publications

1. Pung, Oscar J., and Raymond E. Kuhn. 1987. Experimental American Leishmaniasis in the Brazilian Squirrel Monkey (*Saimiri sciureus*): Lesions, Hematology, Cellular and Humoral Immune Responses. J. Med. Primatol. **16**: 165-174.

2. Pung, Oscar J., and Raymond E. Kuhn. 1988. Experimental Chagas' Disease (*Trypanosoma cruzi*) in the Brazilian Squirrel Monkey (*Saimiri sciureus*): Lesions, Hematology, Cellular and Humoral Immune Responses. Int. J. Parasitol. **18**: 115-120.

3. Pung, O. J., L. H. Hulsebos, and R. E. Kuhn. 1988. Experimental American Leishmaniasis and Chagas' Disease in the Brazilian

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Squirrel Monkey: Cross Immunity and Electrocardiographic Studies of Monkeys Infected with *Leishmania braziliensis* and *Trypanosoma cruzi*. Int. J. Parasitol. **18**: 1053-1059

REPORT

Three studies were completed and published regarding single or mixed infections with New World leishmania species and/or Chagas' disease. All of the studies were funded by the U.S. Army Medical Research and Development Command. These related directly to defining the squirrel monkey as a model non-human primate for use in studies on the immunology of the kinetoplastids.

In the first study, unulcerated cutaneous lesions appeared and persisted in squirrel monkeys experimentally infected with *Leishmania braziliensis braziliensis* or *L. b. panamensis*. Peripheral blood mononuclear cell (PBMC) numbers increased following infection, and cultured PBMCs from infected monkeys proliferated in response to parasite antigens. The responses of PBMCs to mitogens were not suppressed in infected monkeys. Elevated levels of leishmania-specific immunoglobulins M and G were also observed. Thus, the squirrel monkey is susceptible to American leishmaniasis and is capable of responding to the infection with measurable cellular and humoral immunity. Pung, O. J. and R. E. Kuhn. 1987. J. Med. Primatol. 16: 165-174)

Similar studies were performed with monkeys infected with *Trypanosoma cruzi*. Adult, laboratory-bred squirrel monkeys were infected with blood-form trypomastigotes of *T. cruzi* (Brazil strain) and examined during the course of infection for effects on the number and reactivity of peripheral blood mononuclear cells (PBMC) electrocardiographic alterations, and parasite-specific antibody responses. Infection resulted in electrocardiographic changes which included prolonged QRS intervals and QRS axis shifts, possibly indicative of mild intraventricular septal conduction defects or ventricular hypertrophy. Numbers of PBMCs increased slightly while red cell counts, peripheral neutrophil counts and hematocrits were unaltered. PBMCs from infected monkeys proliferated *in vitro* in response to *T. cruzi*, but not to *Leishmania spp.* antigens, with peak responses occurring 15-19 weeks after infection . PBMC responses to various mitogens were not affected by infection. Elevated levels of anti-*T. cruzi* IgM antibodies were detected 1-31 weeks after infection. Anti-

T. cruzi IgG was detected on weeks 6-31. Pung, O. J., L. H. Hulsebos and R. E. Kuhn. 1988. Int. J. Parasitol. 18: 115-120.)

For effects of sequential infections, laboratory-bred squirrel monkeys previously infected with either *Leishmania braziliensis braziliensis* or *L. b. panamensis* were challenged with blood-form trypomastigotes of *T. cruzi*. Monkeys perviously infected with *T cruzi* were challenged with *L. b. braziliensis*. Monkeys were examined during the course of challenge for evidence of infection, electrocardiographic alterations and parasite-specific antibody responses. *T. cruzi* epimastigotes were cultured from the blood of monkeys up to 3 months after challenge with this parasite. Unulcerated cutaneous lesions appeared and persisted in monkeys challenged with *L. b. braziliensis*. The formation of satellite lesions was observed in one monkey. Increased QRS intervals were not observed in *T. cruzi*-challenged monkeys without prior cardiac irregularities and QRS left axis shifts were observed in only two of these monkeys. Elevated titers of parasite binding IgM and IgG specific for both *T. cruzi* and *L. braziliensis* were observed in all monkeys following challenge. These results indicate that prior infection with *T. cruzi* or *L. braziliensis* does not protect against heterologous challenge infection with these organisms. However, prior infection with *Leishmania* parasites may provide some protection against chagasic cardiopathies. Pung, O. J., L. H. Hulsebos, and R. E. Kuhn. 1988. Int. J. Parasitol. 18: 1053-1059.)

A major goal of the study was to determine the nature of antigens of *Leishmania spp.* and *T. cruzi* which could be used to discriminate between leishmaniasis and Chagas' disease and possibly the species of infecting *Leishmania*. To accomplish this, laboratory-bred squirrel monkeys were infected with *L. b. braziliensis* (LBB) or *L. b. panamensis* (LBP). Following recovery from infection they were challenged with *T. cruzi* (TC). Specific *Leishmania* and TC antigens capable of inducing antibody were examined using Western blot analyses. Briefly, soluble LBB and LBP promastigote and TC epimastigote antigens were resolved by SDS-polyacrylamide gel electrophoresis and then electrophoretically transferred onto polyvinylidene difluoride membranes. Membranes were incubated in monkey plasma obtained at various intervals before and after primary and challenge infections. The binding of monkey antibodes in the plasma to parasite antigens was detected using HRP-conjugated rabbit anti-squirrel monkey IgG (heavy and light chain specific).

Pre-infection plasma from the squirrel monkeys contained antibodies capable of binding LBB and LBP antigens. Although particular antigens generally varied from monkey to monkey, a 68,000 dalton antigen of LBB was recognized by antibody in pre-infection plasma from three of the monkeys.

Following primary infection with LBB or LBP, the number and intensity of bands which appeared increased over pre-infection levels. When post-infection plasma was incubated with either LBB or LBP antigen as many as 30 bands appeared in immunoblots ranging in M_r from 13,000 to 200,000 daltons. Peak numbers and intensities of bands occurred approximately four months after LBB infection and 6-9 months after LBP infection. The number of bands and their intensities declined to pre-infection levels 9-10 months after infection in most animals (generally after lesions had resolved).

When plasma from monkeys infected with LBB was reacted with LBB or LBP antigens certain antigen bands were observed in immunoblots which did not appear in immunoblots of either antigen reacted with plasma from LBP-infected monkeys. The antigens were as follows: LBB antigens: 66,000, 34,000 and 18,000 daltons; LBP antigens: 66,000 and 48,000 daltons. Similarly, plasma from LBP-infected monkeys reacted with one LBB antigen of 70,000 daltons and one LBP antigen of 71,000 daltons which were not observed when these antigens were reacted with plasma from LBB-infected monkeys. These antigens may be of some value in differentiating strains of LBB and LBP.

However, a large number of the bands which appeared when LBB and LBP antigen were reacted with primary infection plasma, including those descred above, reappeared when these monkeys were subsequently infected with *T. cruzi*. (The peak number of LBB and LBP antigen bands occurred 1-3 months following TC challenge.) Consequently, the impact of sequential or simultaneous heterologous infection, which probably is not uncommon in people living in endemic areas, should be taken into account during the selection of diagnostically relevant antigens.

With regard to TC antigen, we observed that plasma from monkeys infected with LBB or LBP reacted with 5-13 different TC antigens. When these monkeys were challenge-infected with TC, the number of TC antigen bands appearing in immunoblots increased to as many as 20. Due to monkey to monkey variability in terms of the number and M_r of bands which appeared after primary and challenge infection, no new bands common to all monkeys were observed after challenge.